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GUEST EDITORIAL

Cancer and Complexity: Correlations and Complementarity

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PLEIOTROPY, POLYGENES, AND QUANTITATIVE GENETICS

With the rediscovery of Mendelian genetics at the turn of the century, the idea took hold that individual genes determined single characteristics. In the following years, however, it was found that many, if not most genes, are involved in shaping many different properties of the organism, a condition called pleiotropy. It was, of course, discovered much later that genes coded for individual proteins, as first expressed in the one gene-one enzyme concept, but a single enzyme would be needed for metabolic functioning in a variety of biological processes in the cell. It also became evident that major characteristics of organisms, such as size, disease resistance, egg and milk production, etc., involve many genes, so-called polygenic inheritance. Mutations in polygenic characteristics produced graded or partial effects rather than the quantal effects like color change in peas identified with Mendelian genetics. Analysis of polygenic characteristics required the introduction of quantitative genetics which dealt with continuous variation and was essentially statistical in nature. By the 1920s it was realized that polygenic phenotypes were highly sensitive to developmental perturbations, environmental conditions, and also the modifying effects of the entire genome. At one extreme, a particular gene mutation that is deleterious in one genetic milieu could be beneficial in another.

Given the limited number of multicellular organisms that could be used in classical genetic crosses, it was difficult if not impossible to derive an accurate determination of the number of genes involved in determining particular traits. In the latter half of the century, however, microorganisms became available for genetic analysis, and many millions of them could be used to obtain meaningful results within days. It was then realized that such a seemingly simple process as spore formation in bacteria required cooperative interactions among approximately 1,000 genes [1]. In an even simpler case, the rate of penicillin production in molds was significantly influenced by mutations in any one of some 1,000 genes [2]. The number of possible combinations of mutational effects on these properties in different environments is immense, i.e., $>>10^{100}$, and therefore, in mathematical terms, transcalculational.

As improved methods for genetic and molecular analysis were adopted for multicellular organisms, it was found that the formation of structures in the fruit fly such as the compound eye or the egg involved 70–75% of the genome [3], or close to 10,000 genes. The number of possible phenotypes resulting from genetic variation in such situations is so large that establishing a complete chain of physicochemical causality from genotype to phenotype is not possible. Just as there are limiting laws in physics such as quantum indeterminacy, the impossibility of a perpetual motion machine or of an object moving faster than the speed of light, so there are limits to molecular determinism in genetics and development.

POLYGENIC DELETIONS IN CANCER

A similar analysis of limits can be applied in cancer research as a result of genetic studies from the last few decades. In the 1950s, research with tumor viruses indicated that a single altered gene could cause the neoplastic transformation of a cell and all its descendants leading to development of a tumor [4]. However, pathologists already understood that naturally occurring malignancies usually progress through several stages [5], meaning that more than one gene was involved. Estimates were made that mutations in 5–7 genes were needed for full progression to the malignant phenotype. At the same time, it was realized that the cellular population of a tumor was heterogeneous [6,7], and therefore the genotypes of cells in

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a tumor were likely to be diverse even though the tumor may have initially arisen from a single cell [8].

As molecular and cytogenetic methods for analyzing DNA and chromosomes were refined, more and more genetic lesions were found in solid epithelial cancers. One molecular method detects loss of heterozygosity (LOH) in informative (heterozygous) genes. That technique has disclosed that an average of more than 25% of the informative loci in sporadic colon cancer suffer LOH [9,10]. LOH is usually interpreted as an indication of mutation in a tumor suppressor gene. However, the method used to detect LOH indicates physical loss of the chromosomal segment containing the gene, and frequently of large adjacent regions of the chromosome. Similar findings have now been made in other types of sporadic cancer so it appears that a very widespread disruption of the genome occurs in the most common types of human cancer.

There has been a tendency to interpret development of human tumors in terms of a limited number of specific oncogenes and tumor suppressor genes. Such specificity becomes difficult to justify when a large fraction of the genome is radically altered in development of a tumor. Just as pathologists decided a long time ago that no two tumors are morphologically or behaviorally identical [11], so molecular biologists find that no two solid tumors are genetically identical [9]. In retrospect, this is not surprising in view of the uniqueness of every person's genome and the sensitivity of polygenic phenomena to the microenvironment and to the entire genotypic milieu of the individual.

COMPLEMENTARY METHODS FOR UNDERSTANDING COMPLEXITY IN CANCER

It is against this background that Waliszewski et al.'s [12] article on complexity in cancer has to be considered by oncologists and surgeons. The authors make a detailed theoretical analysis framed in terms of complexity, chaos, fractals, etc., in which they conclude that a deterministic or ''bijective'' relation between genotype and phenotype of a tumor (or organism) cannot be achieved. This conclusion sets a limit to the capacity of genetic analysis of tumor cells to precisely predict the behavior of the tumor, but it also directs us to a more holistic type of analysis which Waliszewski et al. [12] frame in terms of fractals and chaos theory. They do not suggest abandoning the genetic approach but using it in a complementary way together with higher levels of description and analysis.

Without questioning their recommended approach, I should like to suggest another way of dealing with the problem of complexity that does not require a sophisticated understanding of the mathematics of fractals and chaos. This alternative approach grew out of work on the neoplastic transformation of cells in culture which is directly related to their capacity to produce tumors in experimental animals [13,14]. Although it represents efforts of experimental biologists, the general principles of analysis can be applied by oncologists in their own work. The experimental approach is rooted in a theory of organisms developed by a distinguished theoretical physicist, Walter Elsasser, who was intensely concerned for half a century with the question of whether biology could ever be completely reduced to physics and chemistry. Long before the current interest in biological complexity, Elsasser [15,16] realized that complexity and heterogeneity were essential to any theory of organisms. He concluded, as do Waliszewski et al., that any theory of organisms must go beyond quantum mechanics even though the laws of quantum mechanics are never violated in living systems. His basic premise was that ''an organism is a source (or sometimes a sink) of causal chains which cannot be traced beyond a terminal point because they are lost in the unfathomable complexity of the organism'' [16: p. 37]. Proceeding from this basic assumption, he developed four fundamental principles that are unique to organisms. In doing so he was, in a sense, responding to Heisenberg's conclusion that ''concepts of physics, chemistry, and evolution together will not be sufficient to describe the facts [of biology]. Something has to be added to the laws of physics and chemistry before the biological phenomena can be completely understood.''

The first two of Elsasser's principles are of particular importance in cancer biology. The first is *ordered heterogeneity,* which states there can be order in the large while there is heterogeneity in the small. The cell orders the heterogeneity of its biochemical reactions and the behavior of the cell is ordered by its setting in a tissue or organ, etc. This introduces to oncology the dimension of the local environment of a tumor, which includes hyperplasia, dysplasia, and an increase in permissiveness for tumor growth with increasing age of the host (see below). The second is *creative selection,* which implies for oncology that the large number of possible tumor phenotypes are chosen from an immensely larger number of chemical arrangements possible in a cell, and lead inexorably to the uniqueness of each cancer. Perhaps the most important practical aspect of these two principles in cancer research is the significance they have for dealing with higher order phenomena. ''Conventionally designed experiments cannot teach us anything beyond the fact that organisms are physicochemical systems. If [their] holistic properties are to be verified, a different type of experiment from that conventionally used by physicists and chemists is required . . . [it] asks for correlations between phenomena . . . These are qualitatively novel types of questions to be put to nature'' [16: p. 148].

The meaning of this recommendation is best clarified by a concrete example of its application. I have already

mentioned the large number of deletions found in sporadic epithelial cancers [9,10]. One can decide to focus on any one or a few of the thousands of trees in this forest of deletions, or alternatively ask what is the underlying significance of such widespread disruption. It would be difficult at best to determine which of the deletions are limiting in producing a sporadic human cancer when direct experimental quantitation at each stage of the carcinogenic process is inaccessible in the human, and problematic even in experimental animals. Indeed, the method itself for measuring LOH indicates that all of the many deleted regions make some contribution to the excessive growth since the measurement requires that the great majority of cells in the tumors have suffered each loss; i.e., LOH could not be detected if it occurred only in a minority of cells and did not contribute to selective growth of the tumor. However, there is information from the genetics of somatic cells in culture that points to an easy way to study the dynamics of the relation between large scale genetic changes and neoplastic development. Point mutations within a single non-essential gene like thymidine kinase do not cause a slowdown in the growth of a cell and its descendants [17,18], but deletions of the entire gene and adjacent parts of the chromosome result in a heritable decrease in growth rate [19–21].

If we want to know whether neoplastic transformation is associated with deletions, we take a group of cells that can be readily transformed by imposing physiological conditions known to produce the desired effect. In this way, we are not prejudging the outcome by infecting the cells with a particular tumor virus or oncogene. Such transformation can be achieved with a particular established line of mouse cells by constraining their growth through keeping them in a crowded state for an extended period of time [22]. When they are subcultured, many transformed foci appear, and if the treatment is repeated, most cells in the culture are transformed, i.e., produce multilayered foci of cells when subcultured on a monolayered background of normal cells. Whenever transformed cells are obtained, we find that they are permanently damaged [23], i.e., they and all their descendants grow at a *slower rate at low population density* than their untreated progenitors. This then is an almost certain sign that the pronounced reductions in metabolism and growth produced by crowding have resulted in substantial chromosomal deletions, based on the results from somatic cell genetics described above.

The next level of correlation comes from human cancers, where deletions are the most common lesion found in the most prevalent epithelial malignancies [24]. Cytogenetic and LOH data reveal approximately the same sites of frequent deletions. We can thus build a bridge between impaired growth and transformation in cultured cells, deletions in somatic cell genetics, and the most common genetic lesions in cancer.

SIGNIFICANCE FOR CLINICIANS

Lest we think that deletions occurring at just a few specific loci are the carcinogenic culprits, we find that the type of transformed focus produced in cell culture by each independent transforming event is unique [25]. And if that uniqueness is not readily apparent from visual inspection, when we combine it with the heterogeneity of the reduced growth rate [26], we can say that any of an enormous number of deletions can contribute to progressive transformation if we are dealing with an already susceptible cell which can be defined as preneoplastic. Another subline derived from the same ancestral cells but with a different history of subculture will not undergo transformation under exactly the same conditions that transformed the preneoplastic cell line unless subjected to those conditions repeatedly. This introduces another problem of importance in oncology: the different degrees of inherent susceptibility to cancer of cells and of individuals of different genotypes.

All of which lends credence to Waliszewski et al.'s implication that it is problematical in multigenic phenomena to predict the phenotype of a cell from information about the status of a few genes. It reinforces their recommendation that the holistic nature of cellular phenomena in cancer be investigated at different levels of the hierarchical structure of a cell in a complementary manner, but suggests that the hierarchy should be extended to the cell population and beyond. Their use of the term ''complementary'' resonates with Elsasser's generalization to biology of Niels Bohr's complementarity principle. Bohr coined the term complementarity in recognition of the complementary types of measurement that are necessary to reveal the wave and particle character of light. The generalization of complementarity to biology by Elsasser refers to the relation between physicochemical measurements made on cellular components, which require the destruction of its living state, and those functional observations made on the behavior of the intact living cell or organism. In the example of correlations cited above, the physicochemical measurements were made of LOH from the genomes of somatic cells in culture and human tumors, while the functional observations were made on growth rate and transformation of cells in culture. The reductions of growth rate are symptomatic of genetic deletions, but are more comprehensive than the physicochemical measurements are because they reflect large deletions made anywhere in the genome that do not kill the cell. Only a subset of those deletions results in neoplastic transformation and that subset is presumably different in cells of different genetic composition and dependent on the microenvironment surrounding the cell. The pervasiveness of the heritable reduction in growth rate forces attention to the cellular microenvironment of the potentially transformed cells because it

affects the entire population, not just the cell that initiates the tumor. It is holistic in the sense that it is a response to any of a panoply of chromosomal deletions and differs from conventional genetic studies that focus on single genetic loci found in a small minority population. Both types of measurement correlate, complement, and inform one another.

The correlation of complementary descriptions at different levels challenges some common preconceptions about carcinogenesis and suggests directions for further investigation by clinicians. For example, it is commonly held that probability of mutational change is maximized during replication of DNA, which is no doubt true for conventional intragenic mutations. However, the large deletional types of change associated with neoplastic transformation and solid epithelial cancers may be maximized by cell damage and growth constraint. Cancer of the stomach, e.g., is preceded by atrophy [27] as is cancer of the prostate (also post-sclerotic hyperplasia) [28]. The importance of chronic tissue damage to carcinogenesis has been noted in many quiescent organs such as the pancreas, salivary glands, liver, urinary bladder, and thyroid [29]. Carcinoma of the liver is strongly associated with progressive cirrhosis and nodular hyperplasia which result from repeated liver damage [44]. Where cell damage occurs, as seen in apoptosis or necrosis due to cytotoxic drugs [30] or growth inhibitory conditions [31,32], it is likely that many of the surviving cells have suffered genetic lesions that slow their growth, destabilize them, and increase the possibility of neoplastic transformation. Estrogen-dependent tumors in rats are more likely to progress to estrogen-independent autonomy when they are deprived of estrogen and regress than if constant growth is maintained in the continuous presence of estrogen [33]. Tumor incidence rises sharply with age beyond maturity as the functional capacity and growth rate of cells decline [34,35]. These observations suggest that suboptimal conditions that damage tissue and inhibit cell growth increase the frequency of just those types of genetic changes that promote the development of the most common cancers of man [24]. Other correlations of importance to surgeons are the evidence for damage in normal-appearing and hyperplastic tissues adjacent to and even at some distance from cancer of the urinary bladder [36], breast [37], head and neck [38], colon [39,40], and esophagus [41]. Recent results in rats indicate that the microenvironment of the liver is much more permissive for tumor development when liver cancer cells are inoculated into the liver of old rats than into the liver of young rats [42]. If this can be generalized to other tissues and tumors, it would add to our understanding of the exponential increase in the incidence of the most common tumors with age. These observations support the field concept in the origin of cancer [11,43], and could be of importance in determining the amount of tissue to remove surgically and the likelihood of tumor recurrence. Hopefully, the article by Waliszewski et al. and the discussion it elicits will motivate clinicians to make further observations in human cancer which are complementary to and correlated with those made at the cellular and molecular levels. Such multilevel synthesis should provide a deeper understanding of the cancer problem and how best to manage it.

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